Hemostatic Efficacy of Two Advanced Dressings in an Aortic Hemorrhage Model in Swine

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Background: **An effective hemostatic agent capable of stopping severe arterial bleeding and sustaining hemostasis over a prolonged time is required. The U.S. Army recently distributed fibrin sealant (under an Investigational New Drug–approved protocol) and chitosan dressings among deployed medics for treating severe external hemorrhage on the battlefield. The purpose of this study was to evaluate the efficacy of these dressings, as compared with the standard gauze army field dressing, to provide initial and sustained hemostasis up to 96 hours in a lethal uncontrolled arterial hemorrhage model.**

Methods: **Anesthetized pigs were splenectomized and chronically instrumented for fluid/drug administration and continuous monitoring of vital signs. An infrarenal aortotomy was created using a 4.4-mm aortic hole punch and free bleeding was allowed for 5 seconds. While bleeding profusely, a dressing was applied and pressed into the wound for 4 minutes (occluding the distal flow) and then released. If hemostasis was not obtained, the dressing was replaced with a new one (maximum, two dressings per experiment) with another 4-minute compression. If hemostasis was achieved, the abdomen was closed; the animal was then recovered and monitored up to 96 hours. Initial hemostasis, duration of hemostasis, survival time, blood loss, and other variables were measured.**

Results: **Application of army field dressing (gauze) did not stop the arterial hemorrhage and led to exsanguination of all the pigs (n = 6) within 10 to 15 minutes of the injury. Chitosan dressing produced initial hemostasis in five of seven pigs. However, the dressings failed to maintain hemostasis for more than 1.6 hours (range, 28–102 minutes), resulting in secondary bleeding and death of the animals. Fibrin sealant dressing produced initial hemostasis in all the pigs (n = 6) and maintained hemostasis in five cases, with one failure at 2.2 hours. These pigs resumed normal activities and lived for the 96-hour experiment duration. Computed tomographic images and histologic sections of the aortas from surviving fibrin sealant dressing-treated animals showed formation of pseudoaneurysms and early granulation tissue at the aortotomy site. The posttreatment blood loss, duration of hemostasis, and survival time were significantly different in the fibrin sealant dressing group than the chitosan dressing and army field dressing groups.**

Conclusion: **Both chitosan dressing and fibrin sealant dressing stopped initial arterial bleeding that could not be controlled by the standard army field dressing. However, although the fibrin sealant dressing secured hemostasis for up to 4 days, the chitosan dressing consistently failed within 2 hours after application. There may be a risk of rebleeding for high-pressure arterial wounds treated with chitosan dressings, particularly in situations where definitive care is delayed substantially.**

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Over 90% of combat deaths occur on the battlefield before the injured can reach definitive casualty care. The majority of these deaths are caused by hemorrhage.[1] In previous conflicts, nearly 90% of all cases of exsanguination resulted from torso injuries, which are extremely difficult to control using the standard techniques of pressure dressing, tourniquets, and clamping.[2–4] On today's battleground, emphasis on the use of body armor protection may reduce the number of casualties in this category, and hemorrhage from the extremities, head, and neck will account for a greater percentage of deaths.[3] Unlike torso injuries, these injuries can more often be controlled by direct pressure. Information from the Wound Data and Munitions Effectiveness Team database suggests that exsanguination from extremity wounds accounts for more than half of the potentially preventable deaths in combat.[1,5] Thus, hemostasis research and the development of an effective method for treatment of compressible hemorrhage from extremity wounds has become a major priority in combat casualty care research programs.

Nearly a decade of collaborative research between the U.S. Army and other research institutes supported by the Department of Defense has resulted in the development of two efficacious hemostatic dressings. The first one is known as the fibrin sealant dressing, and was developed by the American Red Cross and U.S. Army scientists.[6] The product is primarily composed of clotting proteins purified from

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pooled human plasma from donated blood. The current 10 × 10-cm dressing design consists of two outer layers of human fibrinogen (13.5 mg/cm^2) and a middle layer of human thrombin (40 units activity/cm^2) and $CaCl_2$ (75 μg/cm^2), freeze-dried onto an absorbable Dexon mesh backing. The dressing is manufactured sterilely and treated in two steps (solvent detergent and dry heat) for viral inactivation.[7] On contact with blood, the dressing's clotting proteins dissolve in plasma and an enzymatic reaction of thrombin with fibrinogen forms a fibrin layer that adheres tightly to the injured tissue, stopping the hemorrhage. The hemostatic efficacy of this dressing has been shown in a number of experimental models involving traumatic injuries in large animals.[8–14] The fibrin sealant dressing is classified as a biological and must be tested for safety and efficacy in clinical trials before receiving final U.S. Food and Drug Administration (FDA) approval.

The second hemostatic product, the chitosan dressing, was recently developed by the Oregon Medical Laser Center. Unlike the fibrin sealant dressing, the chitosan dressing is considered a hemostatic device and received FDA clearance for human use without clinical trial in November 2002. Chitosan is derived from deacetylation of chitin (poly-β[1,4]-acetyl-D-glucosamine), one of the most abundant natural carbohydrate complexes present in shellfish. It is a nontoxic, biodegradable molecule with strong mucoadhesive properties. The current 10 × 10-cm dressing design includes a thin, approximately 2-mm layer of freeze-dried chitosan and a nonabsorbable backing material to aid application. On contact with blood, it stops bleeding by absorbing water and transforming into an adhesive material that binds tightly to most underlying tissues. This dressing is indicated for hemostatic treatment of external bleeding only. The hemostatic efficacy of a prototype of the chitosan dressing was demonstrated in a grade V liver injury model in large animals.[15] In this model of primarily high-flow venous bleeding, the dressing was found to be more effective in reducing blood loss and preventing death than standard gauze. In another study using a lethal groin hemorrhage model in pigs, Alam and colleagues found that the addition of chitosan dressings to standard treatment reduced mortality rates by 28% when compared with standard treatment alone.[16]

The U.S. Army continues to support the development and refinement of a dry, ready-to-use, hemostatic dressing suitable for treating combat injuries. The dressing must be effective against severe bleeding that would otherwise lead to exsanguination and must sustain hemostasis for at least several hours to permit safe evacuation of casualties to definitive care centers. Future combat scenarios suggest that dispersed, nonlinear conflicts, including urban battles, may delay evacuation of Special Operation Forces casualties for up to 72 hours. The objectives of this study were to compare the efficacy of the fibrin sealant dressing to the efficacy of the chitosan dressing to stop severe arterial hemorrhage that cannot be controlled with standard gauze dressing and to determine the duration of secure hemostasis and the ability of each dressing to prevent secondary bleeding death in a conscious ambulatory subject.

MATERIALS AND METHODS

Chitosan dressings (HemCon Bandage) were either generously donated by the manufacturer (HemCon, Inc., Tigard, OR) or obtained from the U.S. Army Medical Materiel Development Activity in Fort Detrick, Maryland. Fibrin sealant dressings were also provided by the U.S. Army Medical Materiel Development Activity. These dressings were manufactured by CSL Bioplasma (Victoria, Australia) for the American Red Cross.

Immature female Yorkshire pigs, weighing 37.2 ± 3.3 kg were obtained from a local class A dealer (HDH Swine Farm) and randomly divided into three groups. Animals were housed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care and quarantined for at least 1 week before use. All animals received care in strict compliance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The study protocol was approved by the Animal Care and Use Committee of the U.S. Army Institute of Surgical Research.

Surgical Preparation and Instrumentation

Preoperation screening blood samples were collected during the quarantine period. Complete blood counts (CBCs), coagulation profiles (prothrombin time, activated partial thromboplastin time, and fibrinogen), and serum chemistries were measured to ensure complete health status of the animals. Pigs were fasted for 24 hours before the surgical procedure and had free access to water. On the day of surgery, a venous blood sample was collected to verify normal CBC values. The animals were premedicated with glycopyrrolate (Robinul, 0.01 mg/kg) and a combination of tiletamine and zolazepam (Telazol, 4–6 mg/kg) intramuscularly; initial anesthesia was produced with 5% isoflurane using a face mask. The pigs were then intubated and placed on a positive-pressure ventilator, and a surgical plane of anesthesia was produced by ventilation with 2% to 3% isoflurane in 100% oxygen. The animal's core temperature was monitored with a rectal probe and maintained at 37° to 39°C with water-circulating heating pads. To monitor renal function and urine output during the operation and recovery (up to 24 hours), a Foley catheter (10 Fr, Sherwood Medical) was placed in the bladder transurethrally in the female pigs. This noninvasive bladder cannulation is only possible when female pigs are used. The male's spiral urethra obstructs Foley catheter insertion.

Surgical Procedures

All the surgical procedures were performed using standard aseptic methods. The ear vein was cannulated with a Teflon catheter (21-gauge), and lactated Ringer's (LR) solution was administered (5 mL/kg/h) throughout the operation

to compensate for fluid evaporation. The left femoral artery and vein were cannulated with 48-inch extension tubes for daily arterial blood sample collection and intravenous drug and fluid infusion. The ends of the catheters were tunneled subcutaneously, exteriorized between scapulae, and secured. A telemetry device (TL 11M2-D70-PCT, Data Sciences International) was implanted in the right groin of each pig to remotely monitor vital signs. The telemetry gel-filled catheter (outside diameter, 1.4 mm; inside diameter, 1.1 mm) was placed nonocclusively into a small branch of the right femoral artery, and the electrocardiogram leads were tunneled subcutaneously and secured on the left thigh and right side of the chest. The telemetry device and accompanying equipment (receiver and computer system) permitted 24-hour monitoring and recording of blood pressure, heart rate, electrocardiogram, and body temperature of the animals without restricting their movement.

Next, laparotomy was performed and the pig's spleen was removed. To offset the splenic blood loss, the spleen was immediately weighed and the animals were infused intravenously with warm LR solution at a volume that was three times the organ weight. The splenectomy procedure was performed to enable us to measure small changes in hemoglobin or hematocrit levels and detect slow internal bleeding in recovering animals. This was necessary because the pig spleen is a large contractile organ storing 20 to 25% of the animal's total red blood cells. In the event of hemorrhage, this organ is able to contract and autotransfuse the animal, replacing the red blood cells and maintaining hematocrit at a constant level, precluding diagnosis of small internal bleeding. Next, above the bifurcation, a 10-cm section of the infrarenal aorta was carefully exposed and prepared for cross-clamping. A 10-minute stabilization period was allowed for continuous monitoring of hemodynamic parameters. During this baseline period, a core temperature of 37° to 39°C and a stable mean arterial pressure (MAP) of 60 mm Hg or higher were mandated. At the conclusion of baseline, an arterial blood sample was collected and the preinjury levels for CBC and blood gases were determined.

A reproducible aortotomy based on a previously described model[11] was performed. The infrarenal aorta was cross-clamped and the aortotomy was created using a 4.4-mm-diameter aortic punch. The clamps were then removed and the wound was permitted to bleed freely for 5 seconds. The blood loss during this period was deflected back into the peritoneal cavity and measured as the pretreatment blood loss. While the aorta was bleeding, a dressing was applied through the residual pool of blood and manually compressed against the wound with sufficient pressure to occlude aortic blood flow; distal pressure at the femoral artery level was nonpulsatile and below 15 mm Hg. After a 4-minute compression, hemostasis was visually checked; if hemostasis was not achieved or if rebleeding occurred within the next 10 minutes, the dressing was removed and replaced with a new dressing of the same type, with an additional 4-minute compression. This was done to reduce the likelihood of failure of the dressing resulting from misapplication because it was placed almost blindly in an actively bleeding wound filled with blood. The second dressing was applied under the same conditions as the first one. If hemostasis did not occur after the second attempt, hemorrhage was allowed without any additional intervention until the animal exsanguinated. This event was recorded as a failure of the dressing to achieve initial hemostasis; time of death was recorded once MAP and end tidal P_{CO_2} fell below 20 mm Hg and 15 mm Hg, respectively. The blood and blood clots were collected from the peritoneal cavity and weighed, and the volume of posttreatment blood loss was determined. If hemostasis was achieved and was stable for 10 minutes, the animal was resuscitated intravenously with warm LR solution (three times the volume of pretreatment blood loss at 100 mL/min) and the abdominal incision was closed in layers. A postoperative arterial blood sample was collected for measurements of CBC, blood gases, and lactate level. The dead space of the catheters was filled with heparinized saline and the catheters were protected with a nylon mesh jacket placed on the pig. Anesthesia was discontinued and the animal was extubated.

Postoperative Care and Monitoring

After surgery, animals with stable hemostasis and normal blood pressure were placed in a large metabolic cage and transferred to the animal intensive care unit for recovery and continuous monitoring for 96 hours. Prophylactic antibiotics (Naxcel, ceftiofur sodium) were given intramuscularly once per day for 3 days. Postoperative pain was relieved by twice-daily intramuscular injections of buprenorphine (0.05 mg/kg) for 4 days. Midazolam (0.4 mg/kg) was also prepared for immediate intravenous injection to relieve any potential stress and discomfort associated with conscious aortic hemorrhage in case of sudden dressing failure. Sudden drops in arterial blood pressure along with an increase in heart rate were perceived as symptoms of dressing failure and internal bleeding. If dressing failure occurred, the event was recorded and confirmed by hematocrit measurement and necropsy of the animals at a later time. Arterial blood samples were collected daily and CBC was measured to detect any possible slow bleeding that could not be detected from monitoring the vital signs.

Termination

The surviving animals were anesthetized with an intravenous bolus of midazolam (2 mL, 5 mg/mL) and ketamine (2 mL, 100 mg/mL) and prepared for computed tomographic (CT) scanning. To scan the aorta, 75 mL of a contrast agent (Conray, Mallinckrodt, Inc., St. Louis, MO) was infused into the venous line at 3 mL/s. During this infusion, the lower portion of the pig's body was imaged for approximately 20 seconds at 32 slices per second (Toshiba Aquilion 1b multislice scanner). The animal was subsequently killed with an overdose of barbiturate, and the operated site was carefully

Table 1 Baseline Blood Parameters and Animal Characteristics

Variable	Mean ± STD
Body Weight (kg)	37.24 ± 3.4
Body Temp (C°)	37.7 ± 0.48
Hematocrit (%)	32.1 ± 1.9
Hemoglobin (g/dl)	11.04 ± 0.6
Platelets (1000/μL)	378 ± 115.4
PT (sec)	9.7 ± 0.24
aPTT (sec)	15.9 ± 1.88
Fibrinogen (mg/dl)	219 ± 80
pH	7.46 ± 0.02
Preinjury MAP (mmHg)	68 ± 9.5

exposed and inspected for secondary bleeding, tissue adhesion, and residuals of the dressings. The repaired segment of the aorta with the attached dressing was isolated and recovered for histologic examination. Necropsy was also performed on the animals that died prematurely to verify that dressing failure and secondary bleeding was the cause of death. On a few occasions, pigs were scanned shortly after surgery to determine the status of the repaired site.

Statistical Analysis

The Tukey-Kramer and analysis of variance statistical tests were used to compare the groups for median body weight, coagulation parameters, and preinjury MAP. The Kruskal-Wallis test (nonparametric analysis of variance) was used for comparison of pre- and posttreatment blood loss. Dunnett's multiple comparison test was used as the posttest to compare pairs of group means. The comparisons of survival times and the duration of hemostasis curves were performed using the log-rank test. The incidence of initial hemostasis was compared using Fisher's exact test. Data in the tables are expressed as mean ± SD. Statistical significance was assigned at a greater than 95% confidence level ($p < 0.05$).

RESULTS

Body weight, temperature, hematologic measurements, and preinjury MAP are listed in Table 1. All the parameters except body weight were similar among treatment groups. Although the subjects were randomly distributed, a significant difference was found in animal weight among groups ($p = 0.024$). The average body weights for army field, chitosan, and fibrin sealant dressing groups were 39.7 ± 2.9, 34.9 ± 2.2, and 37.5 ± 3.5 kg, respectively. This difference did not impact the hemostatic function of any of the products. The size of the abdominal aorta was similar across groups, and the hemostatic results of the dressings (success or failure) were not correlated with the weight of the animals. The blood loss data were also normalized on the basis of the body weights.

The overall results are summarized in Table 2. Application of two consecutive army field dressings (gauze) with 4-minute compressions each time did not produce hemostasis. All animals treated with the gauze bled to death shortly after reestablishment of blood flow. These animals did not receive fluid resuscitation because gauze application did not produce initial hemostasis. Figure 1 shows a representative example of the MAP at the femoral artery level and the pre and posttreatment blood loss for this group.

The efficacy of the chitosan dressing was tested in seven animals, among which initial hemostasis was achieved in five. These results showed greater efficacy of chitosan dressing to stop arterial bleeding compared with gauze treatment ($p = 0.02$). A total of 11 dressings from a single manufacturing lot were tested, of which five (45%) were successful and stopped hemorrhage. Hemostasis for the chitosan dressing was time limited, and dressings failed at 28, 43, 81, 87, and 102 minutes after application. Nonetheless, overall survival time was significantly longer in chitosan-treated animals ($p = 0.002$) than in the controls. There was no difference in posttreatment blood loss between these two groups. Figure 2 shows a typical example of the blood pressure profile, pre- and posttreatment blood loss, and resuscitation of

Table 2 Outcomes for Treatment of a Severe Arterial Hemorrhage with Different Hemostatic Dressings in Swine

Dressing Type	Number of Animals	Number of Dressings Used	Pre-treatment Blood Loss (mL/kg)	% Initial Hemostasis Achieved	Post-treatment Blood Loss (mL/kg)	Duration of Hemostasis (hrs)	Survival Time (hrs)
Gauze (AFD)	6	12	6.6 ± 2.6	0 (0/6)	39.8 ± 8.5	0	0
Chitosan	7	11	7.1 ± 2.3	71 (5/7)[1]	35.3 ± 8.0[3]	0.81 ± 0.7[5]	0.97 ± 0.7[7]
Fibrin Sealant	6	7	8.4 ± 1.1	100 (6/6)[2]	5.6 ± 13.6[4]	80.4 ± 38.2[6*]	80.4 ± 38.1[8*]

[1] vs. gauze, $P = 0.02$.
[2] vs. gauze, $P = 0.002$; vs. chitosan, not significant (NS).
[3] vs. gauze, NS.
[4] vs. gauze, $P < 0.05$; vs. chitosan, NS.
[5] vs. gauze, $P = 0.008$.
[6] vs. gauze, $P = 0.0005$; vs. chitosan, $P = 0.004$.
[7] vs. gauze, $P = 0.002$.
[8] vs. gauze, $P = 0.0008$; vs. chitosan, $P = 0.004$.
* Data were truncated at 96 hrs for 5 surviving animals.

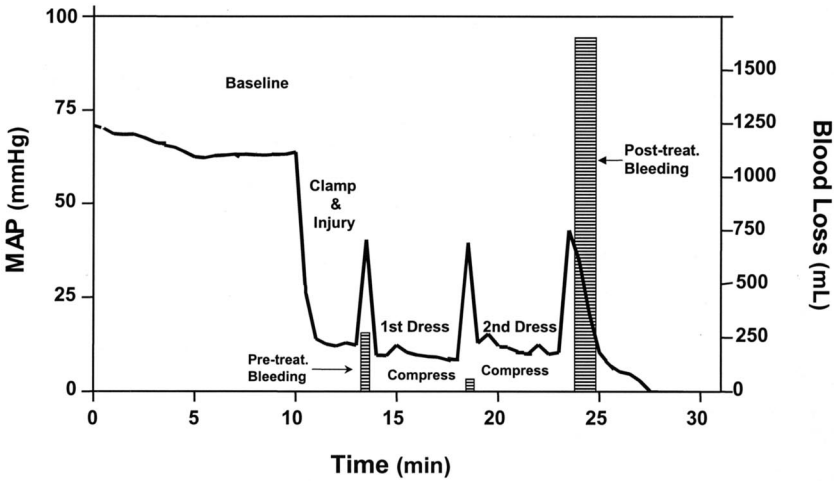

Fig. 1. *Profile of the femoral artery mean arterial pressure and pre- and posttreatment blood loss of a typical animal treated with standard army field dressing (gauze). Hemostasis was not achieved by this treatment and the animal exsanguinated 15 minutes after the injury.*

a chitosan-treated animal. Note the positive blood pressure response to fluid resuscitation, and the sudden drop of pressure at the 60th minute (42nd posttreatment) that was caused by dressing failure and secondary bleeding.

The fibrin sealant dressings produced initial hemostasis in all six animals tested ($p = 0.002$ vs. gauze). A total of seven dressings was used in this group, of which six were effective and stopped arterial hemorrhage. There was a trend for a higher percentage of fibrin sealant dressings than chitosan dressings to be efficacious (86% vs. 45%, $p = 0.15$). Figure 3 illustrates the blood pressure profile of a typical fibrin sealant pig. The fibrin sealant dressing remained in place and prevented secondary bleeding despite a substantial rise in blood pressure after full recovery of the animal. Hemostasis was sustained in the majority of animals treated with the fibrin sealant dressing (five of six) for the 96-hour duration of the experiment. One dressing failure occurred 2.2 hours after application, causing exsanguination. All surviving animals recovered well and resumed normal activities (e.g., eating, drinking) 24 hours after the operation. Blood chemistry values, hemoglobin, hematocrit, and platelet counts remained within normal ranges during the 4-day follow-up period. Hemostasis duration (Table 2) and survival time (Table 2 and Fig. 4) were significantly longer for the pigs treated with fibrin sealant dressing than the other two groups ($p \leq 0.004$). The average posttreatment blood loss in this group was also significantly less than that of the control group ($p < 0.05$).

Gross examination of the wound and the fibrin sealant dressing 96 hours after treatment showed an almost intact dressing, with no degradation and no significant tissue adhesion to the backing material. Dressings appeared to be at-

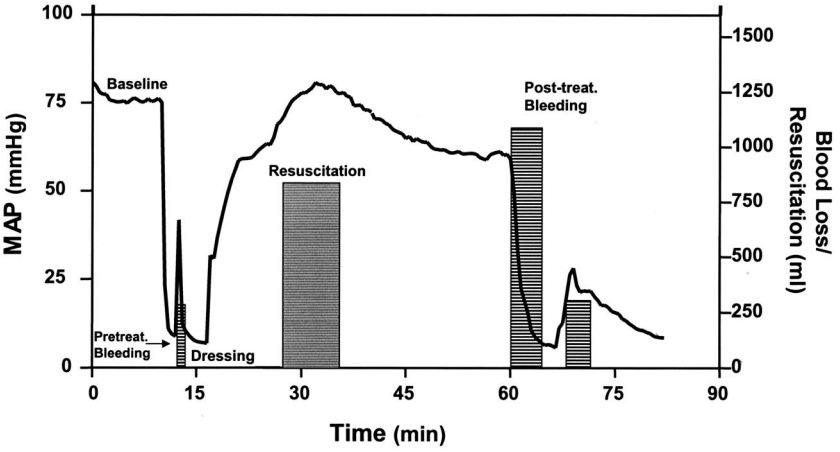

Fig. 2. *Profile of the femoral artery mean arterial pressure, pre- and posttreatment blood loss, and resuscitation of a typical pig treated with chitosan dressing. Hemostasis was achieved with the application of the first dressing and lasted for approximately 45 minutes. The animal died after two rebleeding episodes caused by dressing adhesive failure. Note that the rebleeding occurred when the pressure was below baseline level; therefore, the failure could not be attributed to the recovery of the animal and a rise in blood pressure.*

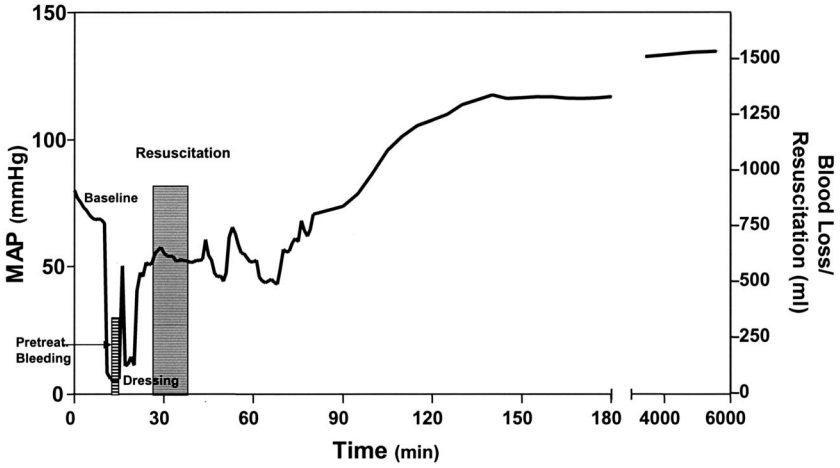

Fig. 3. *Profile of femoral artery mean arterial pressure, blood loss at pretreatment, and resuscitation of a typical subject treated with fibrin sealant dressing. Hemostasis was achieved with application of one dressing and lasted for the duration of the experiment (96 hours). Note the substantial rise of blood pressure after anesthesia was discontinued and the animal fully recovered.*

tached tightly to the injured tissues and there was no evidence of secondary bleeding or hematoma formation. CT images obtained within 30 minutes after the surgery and before termination (96 hours postoperatively) showed gradual formation of pseudoaneurysms at the aortotomy site where the dressing was initially placed (Fig. 5). The dressing appeared to be pushed outward and formed a small pocket over the injury site. Blood circulated within the pseudoaneurysm but did not leak out into the peritoneal cavity. Histologic sections of the vessels at the injury sites showed attachment of the fibrin dressing to periarterial fibroadipose tissue on the vessel and the formation of a pseudoaneurysm (Fig. 6*A*). The new aortic lumen was composed of the original tunica intima with endothelium; the edge of the cut through the vessel wall, covered with a thin layer of clotted blood and overlying endothelium; and a variably thick zone of fibrin and blood clot subjacent to the remnants of the dressing (Fig. 6*B* and *C*). There was scant evidence of early granulation tissue at the margins of the clot.

DISCUSSION

This study compared the efficacy of two advanced hemostatic dressings that have been deployed on the battlefield for the treatment of severe external bleeding. The current army field dressing (gauze) served as the control. These dressings were designed to treat exsanguinating external hemorrhages that cannot be controlled by common currently available methods such as tourniquet, gauze, or pressure dressings in prehospital situations. Previously, the efficacy of the fibrin sealant dressing to stop active arterial bleeding and maintain hemostasis for 1 hour was demonstrated in anesthetized animals,[11] but similar information about the efficacy of the chitosan dressing, particularly the scaled-up production HemCon Bandages, has not been available. Hemostasis duration for these dressings was also unknown. The results of this study show that both dressings can stop severe arterial bleeding and prevent acute exsanguination that cannot be controlled by the standard army field dressing. The fibrin sealant dressing, however, exhibited greater efficacy and more consistent performance than the chitosan dressings. It produced hemostasis in all six animals, requiring a total of seven dressings (86% effective), whereas the chitosan dressing produced hemostasis in five of seven animals, for which 11 dressings (45% effective) had to be used. Before these experiments, a pilot test was conducted in the same animal model to screen four different production lots of the chitosan dressing. The apparent best lot was selected from these four groups for application in the current study. Variability in the efficacy of the chitosan dressing in a lethal groin injury model was also reported by Alam et al.[16] Although five dressings

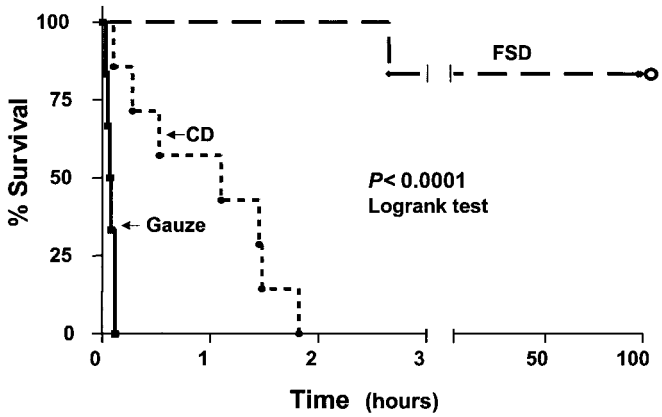

Fig. 4. *Kaplan-Meier analysis of survival data. The fibrin sealant dressing maintained hemostasis and supported survival of animals for a significantly longer period of time than the other products* ($p < 0.001$, *log-rank test). The chitosan dressing was also found to be more effective than the army field dressing (gauze)* ($p < 0.01$, *log-rank test).*

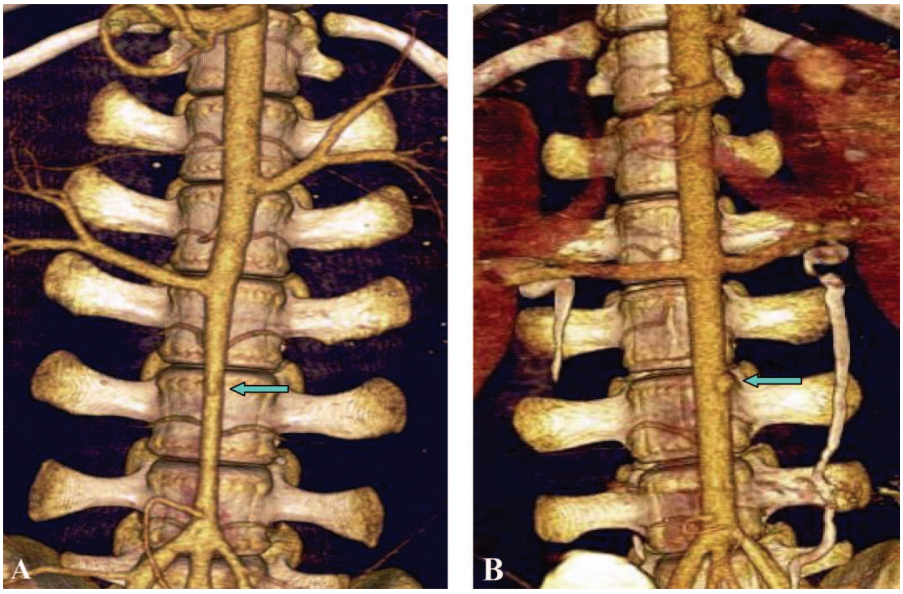

Fig. 5. *CT images of a pig's infrarenal aorta repaired with fibrin sealant dressing shortly after the operation (*A*) and 4 days later at the conclusion of the experiment (*B*). Note formation of a pseudoaneurysm at the aortotomy site at 4 days after surgery. The difference in the diameter of the aorta may be attributable to partial vasoconstriction of the vessel in response to the surgical manipulation.*

stopped hemorrhage effectively and prevented death in that study, two dressings from the same batch failed to produce hemostasis. Moreover, the dressings from another chitosan lot failed entirely in their pilot experiment. These inconsistencies were reported to the chitosan dressing manufacturer (HemCon, Inc.), and as a result, the quality standards for releasing each production lot have now been improved.

Another major difference between chitosan and fibrin sealant dressings was hemostasis duration. Although the fibrin sealant dressing secured hemostasis for up to 4 days in all but one case, the chitosan dressing consistently failed within 28 to 102 minutes after application. As a result, the treated animals bled to death before fully recovering from anesthesia. It was hypothesized that the motion from transporting the animals to the intensive care unit or the accumulation of peritoneal fluid over the dressing might have been responsible for this failure. Therefore, two additional experiments were conducted in which animals were kept under anesthesia on the operating table after treatment, the dressings were fully exposed, and the moisture around the wounds was continuously removed. Despite these measures, the hemostatic dressings failed 28 and 43 minutes after application and the animals bled to death. It was observed that the applied dressings that seemed to adhere to underlying vessel and muscle tissues gradually absorbed moisture, swelled, and became gelatinous. These changes seemed to be associated with a decrease in tissue adhesiveness and eventual hemostatic failure of the product. Although no secondary bleeding occurred in the fibrin sealant dressing animals that were fully recovered several hours after surgery, the CT scans and histologic cross-sections of the aortic segments clearly showed formation of a pseudoaneurysm at the injury site after 96 hours. This condition seemed to occur because the fibrin layer was bound to the collagenous adventitia layer rather than the muscle layer (media) of the vessel. The pulsatile and high pressure of arterial blood flow against the dressing gradually stretched the loose adventitia layer and pushed the dressing outward, causing expansion of the lumen and formation of pseudoaneurysm. The cross-linking between fibrin and a number of adhesive glycoproteins, including fibronectin, von Willebrand factor, and collagen, is responsible for anchoring the fibrin clot to the injured tissues.[17–19] The potential for ruptured pseudoaneurysm or the disappearance of the pseudoaneurysm and consequent healing of the aortotomy is currently under investigation in a long-term fibrin sealant dressing survival study.

The ideal dressing for far-forward military application should meet the following criteria: ability to stop severe arterial or/and venous hemorrhage in less than 2 minutes; easy to apply without the need for premixing agents; biologically safe (sterile and virally inactivated), without any adverse effects; lightweight and durable; long shelf life (2–3 years); functional in extreme temperatures (from −10° to 50°C); biodegradable and fully reabsorbable; and inexpensive. Neither the chitosan dressing nor the fibrin sealant dressing meets all of the desired criteria for military use. Each dressing has its own advantages and disadvantages and may be more suitable for different levels of care. The chitosan dressings are stable, rugged, light, and relatively inexpensive ($100 each), but they are rigid and difficult to apply over a complex wound and have time-limited efficacy. They may be more appropriate for liberal use as a temporary means to control bleeding on the front line. The fibrin sealant dressing is much more pliable after blood contact, conforms and ad-

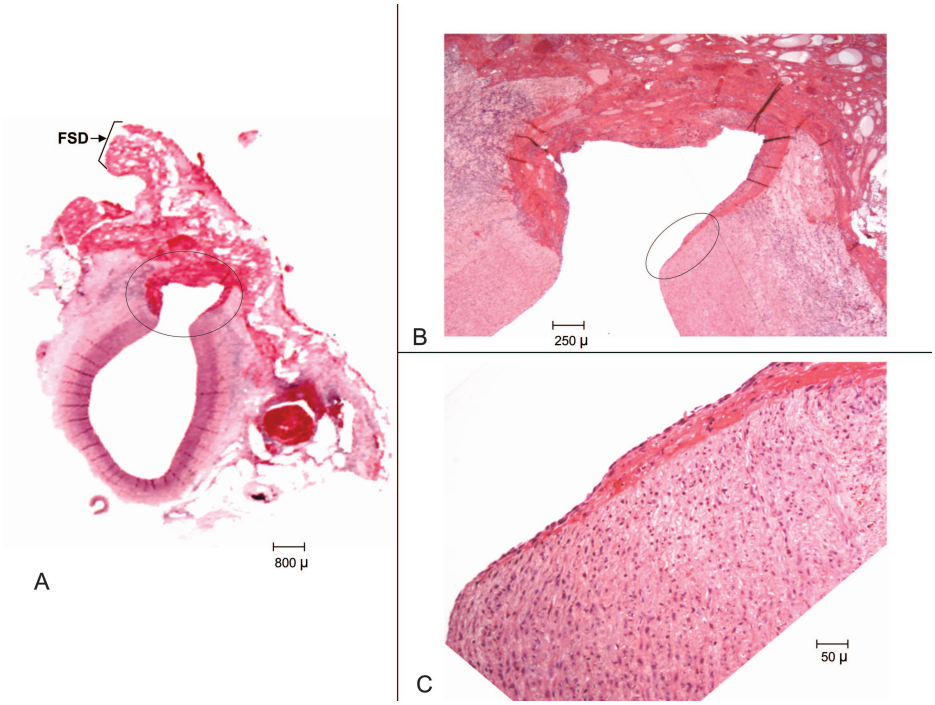

Fig. 6. *Cross-section images of the aorta 4 days after repairing with fibrin sealant dressing (hematoxylin and eosin staining). (*A*) A photograph of the whole vessel and the attached dressing. (*B*; encircled panel* A *area) A micrograph of the aortotomy site with fibrin and blood clots forming a fragile vascular wall and early granulation tissue. (*C*; encircled panel* B *area) Higher magnification of the cut through vessel wall covered with a layer of blood clot and overlying endothelial cells.*

heres well to complex injuries, and is able to stop aggressive, life-threatening bleeding that is currently untreatable by any other means. It is fully reabsorbable and safe for implantation and permanent repair of parenchymal/vascular injuries.[8–10] These dressings, however, are fragile and require special handling and precautions to avoid moisture before application. Their potential cost is likely to be high because of the requirement of a large amount of human fibrinogen and several pathogen removal procedures. The fibrin sealant dressing may be more suitable for use in far-forward surgical units for damage control operations[20] and/or at hospitals to ease definitive surgical interventions and reduce morbidity. In recent military operations in Afghanistan, a limited number of fibrin sealant dressings was distributed among Special Forces medics under an approved Investigational New Drug protocol to treat severe external bleeding that could not be controlled by standard treatment. The dressing was used only once and resulted in a successful outcome. The fibrin sealant dressing has now been replaced by the FDA-approved chitosan dressing, which is widely distributed among U.S. troops. Anecdotal reports coming from the battlefield have been very positive regarding the effectiveness of the chitosan dressing to treat combat-related hemorrhages (LTC Ian S. Wedmore, personal communication).

Recently, an alternative source for coagulation proteins was identified in the blood plasma of farm-raised Atlantic salmon fish. The low temperature of the fish and the evolutionary distance between fish and humans makes pathogen transmission much less likely than it would be from mammalian blood products. Both fibrinogen and thrombin purified from fish plasma can function together with mammalian clotting factors and tissues,[21,22] suggesting that these materials may have utility in fibrin sealant production. There are obvious safety concerns related to clinical use of nonmammalian proteins; therefore, a number of standard immunologic and toxicologic tests are underway to reveal any adverse reactions of these new sources of clotting proteins. A few prototype dressings have been made with lyophilized salmon fibrinogen and thrombin and tested in a swine aortotomy model with encouraging results (S. Rothwell, personal communication).

Another hemostatic dressing, known as the rapid deployment hemostat (RDH), has been derived from algae for control of severe extremity bleeding in military and civilian trauma. The RDH dressing consists of a specific hemostatic formulation of fully acetylated poly-*N*-acetyl glucosamine. Although the exact mechanism of its hemostatic action remains unclear, suggested mechanisms include red blood cell aggregation, platelet activation and activation of the clotting cascade,[23–25] and local vasoconstriction be means of endothelin release.[26] The original RDH dressings were tested in swine models of traumatic injury, including severe liver injury[27] and aortotomy.[11] In both of these lethal hemorrhage models, the RDH was ineffective in reducing blood loss or

increasing survival. Subsequently, the RDH was modified by increasing the active ingredient and adding a surgical gauze backing for better handling and increased efficacy, resulting in the so-called modified RDH. In further testing, the modified RDH was shown to be effective in reducing blood loss and increasing survival in similar models of aortic[28,29] and liver injury in swine.[30] In these successful trials, however, certain procedures (e.g., prolonged compression and the Pringle maneuver) were incorporated into the application of dressing that might have increased the effectiveness of the product. Another formulation of modified RDH dressing did not change blood loss or mortality in a lethal groin injury model of swine.[16] This dressing is an FDA-approved hemostatic device for use on extremity bleeding from trauma. In a recent clinical study, the modified RDH dressing was shown to effectively terminate bleeding in coagulopathic patients with severe visceral injuries.[31] The modified RDH dressing is not yet a commercial product and samples could not be obtained to include in this study.

A fair question about the model used in this study is, why—if the dressings are designed to treat external traumatic bleedings in the prehospital situation—were they tested in an internal arterial hemorrhage model? The primary reason for this is the technical difficulties involved in the design of an external hemorrhage model in a conscious ambulatory animal. For instance, if the dressings were placed on a femoral or a carotid artery injury, the animals would have been able to chew or scratch the dressings, and these potentially confounding factors could not have been ruled out as the cause of product failure. Restraining the animals or deeply sedating them over a long period of time (up to 96 hours) was not feasible. Alternatively, modification of the swine aortic hemorrhage model originally developed in our laboratory[11] provided an ideal condition for testing the long-term efficacy of the dressings. This model offered several advantages: a consistent injury and lethal arterial hemorrhage that could not be controlled by standard gauze treatment; a treated site that was not subject to motion or animal interference; an arterial vessel that was comparable in size to the human femoral artery but less vasoactive and more challenging to the dressings; an experimental condition in which delayed hemostatic failure of the dressing could be easily and precisely identified; and easily quantifiable hemorrhage volume because blood loss was contained in the closed abdomen.

In summary, the present study compared the efficacy of the fibrin sealant and chitosan dressings that were recently deployed on the battlefield for treatment of severe external bleeding. The dressings were tested for their ability to repair a 4.4-mm aortotomy injury in a lethal hemorrhage model in pigs. Their ability to stop the initial hemorrhage and subsequently maintain hemostasis up to 96 hours in conscious ambulatory subjects was tested and compared with the current standard of care (army field dressing). Both test dressings were able to stop the initial aortic bleeding and produce hemostasis that could not be controlled by standard gauze treatment, with a success rate of 71% for the chitosan dressing and 100% for the fibrin sealant dressing. However, the hemostasis produced by the chitosan dressing was unstable and the dressings failed on an average of 49 minutes (range, 28–102 minutes) after application, whereas the fibrin sealant dressing, in all but one case, produced secure hemostasis for 96 hours. These data suggest that there may be a risk of rebleeding for arterial wounds treated with chitosan dressings. Such arterial wounds should be closely observed by medics, and if bleeding resumes, the original dressing should be removed and replaced with a new dressing. The long-term efficacy of the fibrin sealant dressing and its potential application instead of suturing in damage control operations are currently under investigation.

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DISCUSSION

Dr. Hasan B. Alam (Washington, D.C.): Thank you, Dr. Rozycki, Dr. Nakatani, good afternoon members and guests. I'd like to thank the AAST for the privilege of discussing this paper.

It's a timely and well-executed study from a group that has established itself as one of the leaders in the field of hemorrhage control, especially hemostatic strategies to combat injuries.

In this study, investigators created a standardized aortic injury in swine and attempted hemorrhage control with either Army field dressing or two hemostatic dressings. These included fibrin sealant dressing made from pooled human plasma and Heme-compressing made from chitosan derived from shellfish. In addition to the early effectiveness of these strategies, they also evaluated the durability of the hemorrhage control. The standard Army field dressing failed to control hemorrhage in this model; whereas, application of Heme-com worked in five out of seven animals and fibrin dressing worked in all six animals.

The interesting finding of the study was not that heme and fibrin dressings worked well. The effectiveness of this dressing has convincingly been demonstrated in numerous studies. Unfortunately, this agent is unlikely to get FDA approval in the near future. In addition, it's expensive and not very easy to use.

The important discovery was that while the application of Heme-com dressings had an early success rate of 45% during initial hemorrhage control, all of these dressings failed during the observation period, resulting in lethal rebleeds. The time to failure was as early as within the first half of an hour. None of the dressings stayed in place for even two hours. Batch-to-batch wear-ability in the effectiveness of Heme-com has been reported by many, including our own group. But in this study, the researchers had screened four production lots to choose the best products.

I have a couple of comments and a few very simple questions. I think the study was well designed. Choosing the aorta as the site of injury is somewhat artificial, as medics in the field only have access to external sites where the dressing may behave very differently due to the smaller size of the vessel, lesser flow, and decreased hydrostatic pressure. Addition of a lower pressure venous injury would also have strengthened the study. However, these are minor issues. Your findings are of concern, because these dressings have been widely deployed to the troops in the battlefield. The failure of the Heme-com dressing seems to be early, abrupt, and without warning.

Have these findings been shared with the medics that are using the dressings? Has there been any change in the training protocol? Maybe these dressings should be used on soft tissue injuries and venous injuries but not an arterial site.

I think that your recommendation to frequently inspect the dressings after application is not a good idea at all. The hemostatic dressings are typically covered by regular compression dressing, and the usual teaching is not to disturb the dressing after successful hemorrhage control unless evacuated to a higher echelon of care.

Removal of the covering dressing every 30 minutes or so for inspection is not only impractical, but it may actually dislodge the underlying heme contrasting. So my final question is, are there any predictable signs short of renewed hemorrhage that can alert the medic that the hemostatic dressing is about to fail?

Congratulations on a well-done study, on a nice presentation. Once again I would like to thank the AAST for the privilege of the floor. Thank you.

Dr. B. S. Kheirabadi (closing): Thank you, Dr. Alam. As far as the model concern, I do agree; however, we had to design a model that would allow us to address and answer the objective question that we had. Perhaps using the venous model, we were not convinced that it was sufficient to show

if there is actually a failure, that we would be able to recognize it. Besides, the model was such that it would allow us to look at this without the interference of the animal. We would be able to measure and make sure, consistently and precisely, when the dressing failed.

With respect to whether we have shared this information with medics, we are going to present this in our larger meeting, in Army meetings. We have shared this with the manufacturer, Heme-com, and noted that there is variability in the loss, and as a result, they have voluntarily changed their specs for releasing of the product. Now, some of the lot that we have received to screen will no longer be released to the medics, because they will not have been approved, so that change has been made. And the quality standard for releasing the lot has been improved and increased.

They are well aware of this potential problem with the failure of dressing, and initially, we're not sure whether that was because of the fluid in the abdomen and others. We have assured them that is a potential problem with the dressing.

I agree with you in terms of perhaps inspecting the wound, and taking the dressing off of it would be a disadvantage, and it would not be practical. However, by putting the dressing on and wrapping it and not being worried at all also might be a mistake, so frequent inspection in terms of at least making sure there is no rebleeding occurring is an important aspect of it.

There is really no predictable sign to see when this dressing will fail. When you have exposed the dressing, it is exposed; you can see the dressing continuously absorbs the moisture. It swells and turns gelatinous, and that's when it begins to fail. In the battlefield, if it is covered with another gauze, it's hard to know when it's going to fail, and it can fail at any moment.

Again, I thank the society for the purpose. Thank you.